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Separation and characterization of a subset of human T8+ cells which function as antigen-presenting and contrasuppressor cells

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Summary. The lectin *Vicia villosa* (VV) binds preferentially a subset of T cells. Separated VV-adherent cells (AC) do not generate helper function alone, but when added to VV-non-adherent cells (NAC), they can present streptococcal antigen (SA) and induce helper function as effectively as monocytes, at a ratio of 1 VV-AC to 4 VV-NAC. Further separation into a T8+ subset of VV-AC and reconstitution with T4+ helper cells (HC) has established that the T8+ VV-AC induces T4+ cells to helper activity. However, in addition to antigen presentation, the T8+ subset of VV-AC has a contrasuppressor function, for it can prevent T8+ suppressor cells from inhibiting T4+ HC function. The results suggest that a T8+subset can present antigen to T4+ HC, activate helper function and prevent suppression. The T8+ VV-AC may have considerable biological significance in its dual function of countering the dominant suppressor activity and presenting antigen to induce helper activity.

INTRODUCTION

It has been suggested that immunoregulation in the mouse is dependent on cell circuits consisting not only of macrophages, helper, inducer and suppressor cells, but also of contrasuppressor cells (Gershon *et al.*, 1981; Green *et al.*, 1981; Ptak *et al.*, 1981). The contrasuppressor cells (CSC) prevent suppressor cells from inhibiting helper cells in forming antibodies to sheep red blood cells (Gershon *et al.*, 1981) or convert tolerogenic to immunogenic signals in contact sensitivity (Ptak *et al.*, 1981).

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A human subset of T8+ cells has been postulated to function as CSC, on the basis of a parallel study of helper and suppressor functions to a streptococcal antigen (Lehner, 1982). An increasing dose of antigen elicited low-dose and high-dose suppression with an intermediate dose of helper activity. The helper activity was ascribed to a subset of T8+ cells which inhibit suppression to allow helper activity and therefore function as CSC. Indeed, an antigen-binding subset of T8+, T4− cells was identified which binds the same dose of antigen as that required to elicit helper function (Lehner, 1983a). The cell can bind antigen directly at 4° and present it to T4+ HC to induce helper activity (T. Lehner, J. Avery and T. Jones, submitted for publication).

The objective of this study was to isolate or enrich the putative CSC by panning T or T8+ cells with the lectin *Vicia villosa* (VV) used in the separation of mouse CSC (Green *et al.*, 1981). The separated subsets of T or T8+ cells were then characterized for phenotypic markers and functional activities. The T8+ VV-adherent cells can present antigen to T4+ HC, activate helper function and prevent suppression by another subset of T8+ VV-non-adherent cells.

MATERIALS AND METHODS

Cell separation and depletion

Specimens of venous blood were withdrawn from ten

healthy subjects whose HLA-DR was determined as described previously (Welsh & Batchelor, 1978; Lehner *et al.*, 1981). All subjects were HLA-DRw6$^-$; six had DR4 and four had DR2, 3 or 5, as their cells yield helper activity with 1 μg streptococcal antigen (Lehner, 1982). Mononuclear cells were separated by the Ficoll Triosil (FT) method (Boyum, 1968). Adherent cells, predominantly monocytes (Mo), were prepared by adherence of FT separated cells to plastic plates (Falcon 3002; 6 cm diameter) as described elsewhere (Lehner, 1983a). The FT-separated and plastic non-adherent cells were passed through a nylon-wool column to remove B cells and any adherent cells (Julius, Simpson & Herzenberg, 1973). T8$^+$ and T4$^+$ cells were separated in some experiments by killing T cells, either with monoclonal OK.T4 or OK.T8 antibodies (Ortho Laboratories, Raritan, NJ) and rabbit complement (Buxted Rabbit Co Ltd, Sussex), as described previously (Lehner, 1982). The resulting T-cell populations were characterized by indirect immunofluorescence (Lehner, 1982).

Separation of T or T8$^+$ cells by Vicia villosa

The rationale for using the lectin *Vicia villosa* (VV) was that murine CSC express selective binding affinity to VV (Green *et al.*, 1981). The panning method was applied (Wysocki & Sato, 1978) using VV (Sera-Lab, Crawley, Sussex) with high-binding affinity for N-acetyl-D-galactosamine. Falcon plates were incubated with 2·5 ml VV, at a concentration of 0·5 mg per ml in Hanks' solution (pH 6·5), for 2 hr at room temperature. After removal of the unbound lectin, the plates were washed twice with Hanks' solution. T or T8 cells were then added to the plates at a concentration of about 20×10^6 cells in 3 ml Hanks' solution, for 30 min at 37°. The non-adherent cells were removed and the cells adherent to the plate were washed twice with Hanks' solution at 37°. The adherent cells were then recovered from the plate by adding 3 ml N-acetyl-D-galactosamine at a concentration of 2 mg per ml in Hanks' solution, for 10 min at 37°. The cells were then characterized as described below.

Preparation and assay of helper and suppressor factors

All subjects investigated so far have been apparently sensitized by streptococcal antigen (SA) *in vivo* and have helper (Lehner *et al.*, 1981) and suppressor cells to the SA (Lehner, 1982). In order to induce helper factor *in vitro* from the putative helper cells (HC) *in vivo*, 5×10^6 of the depleted or reconstituted cell populations were pulsed with the predetermined dose of 1 μg of SA in HEPES-MEM and 5% fetal calf serum (FCS) for 24 hr in Marbrook flasks. Any helper factor released into the supernatant was assayed at a previously determined optimum concentration of 10 μl per culture in Costar plates (Cambridge, MA) in the presence of 5×10^6 unprimed CBA/CA spleen cells and 100 ng/ml of DNP-SA. The anti-dinitrophenyl (DNP) antibody-forming cells (AFC) were assayed on Day 4, using the modified Cunningham assay (Cunningham & Szenberg, 1968) with trinitrophenyl (TNP)-coated sheep red cells (SRC) and uncoated SRC. DNP-specific plaques were the difference between the two. Since unprimed spleen cells were used, only IgM AFC were detected. All cultures were carried out in triplicate and assayed separately. The helper activity was expressed as the number of antibody-forming cells per culture and the percentage change was calculated by using the following formula:

$$\frac{\text{AFC with helper factor and SA} - \text{AFC with SA}}{\text{AFC with SA}} \times 100.$$

Suppressor factor was prepared from putative suppressor cells in the same way as described for helper factor. The cells were cultured in the presence of 1 μg of SA for 24 hr and the supernatants were then assayed for their ability to suppress helper cells (Lehner, 1982). Helper cells were induced from CBA/CA mouse spleen cells by culturing 15×10^6 cells per ml of HEPES-MEM with 100 ng of SA in Marbrook flasks for 4 days. The suppressor factor (10 μl) was then cultured in the presence of 3×10^5 HC, 15×10^6 unprimed CBA/CA spleen cells and 100 ng of DNP-SA. AFC were assayed on Day 4 as for helper factor. The suppressor factor activity was expressed as a percentage decrease of anti-DNP AFC of the mouse CBA helper cells, cultured for 4 days with the suppressor factor, using the following formula:

Suppressor activity (%) =

$$100 - \frac{\text{AFC with suppressor factor and SA} - \text{AFC with SA}}{\text{AFC with helper cells and SA} - \text{AFC with SA}} \times 100.$$

Preparation of antigens

Streptococcal antigen (SA) was prepared from *Streptococcus mutans* (serotype c, Guy's strain) grown in a semi-defined medium as described previously (Russell *et al.*, 1980). DNP-SA was prepared using dinitrofluorobenzene as reported before (Lamb, Kontiainen & Lehner, 1979) and had five groups of DNP per 100,000 MW. Tetanus toxoid (TT) was purchased from Wellcome Reagents (Beckenham, Kent) and

TNP-TT was prepared by adding 2 mg TT to 2 mg sodium dinitrobenzene sulphonate, and the mixture was stirred in the dark, overnight, at 4°. The solution was then dialysed against PBS for 72 hr, changing the dialysate twice daily. The TNP-TT had 8·6 groups of TNP per molecule of tetanus toxoid.

Characterization of cells

The proportion of cells reacting with OK.T4, T5, T8 and Ia, monoclonal antibodies (Ortho Diagnostic Systems) and goat anti-human F(ab) antiserum (Northeast Biomedical Laboratories, Uxbridge, Middlesex) were assessed by indirect immunofluorescence as described elsewhere (Lehner, 1982). Aliquots of 10^6 cells were incubated with 5 μl of the antibodies for 30 min at 4°, washed twice, and then 5 μl of FITC-conjugated goat anti-mouse IgG (Fc) (Miles Laboratories, Kankakee, IL) or rabbit anti-goat IgG (Northeast Biomedical Laboratories) was added and incubated for 30 min at 4°. After washing twice, the cells were mounted on microscope slides and over 200 cells were counted for membrane fluorescence with a Leitz SM-Lux fluorescence microscope, with a ploem illuminator. Phagocytic cells were assessed by mixing the cells with latex particles coated with human IgG (Wellcome Reagents) and counting the proportion of cells with more than three ingested particles (Lehner, 1982).

The effect of reconstitution of VV-non-adherent cells with VV-adherent cells or Mo on the helper function

VV-NAC were separated from VV-AC by panning T cells as described above, and the cells were tested for helper function with 1 μg SA. The VV-NAC were then reconstituted with VV-AC by adding progressively larger numbers of VV-NAC to corresponding smaller number of VV-AC, to a total of 5×10^6 cells. Similar quantitative reconstitutions were carried out with Mo and VV-NAC. The cells were then cultured in Marbrook flasks with 1 μg SA for 24 hr, and the culture supernatants were used to assess helper activity in the cooperative cultures with mouse CBA-spleen cells as described above.

The effect of reconstitution of T4+ HC with T8+ VV-AC or T8+ VV-NAC on the helper and suppressor functions

In order to increase the purity of SA-presenting T cells, VV-NAC were separated from VV-AC by firstly panning T8+ cells so that both VV-AC and VV-NAC consisted predominantly of T8+ cells. These cells were then reconstituted with T4+ cells, and the effect on the helper and suppressor functions of 1 μg SA were then assessed.

The effect of varying the number of T8+ VV-AC, the dose of SA and the dose of helper or suppressor factor on the corresponding functions

In view of the small number of cells available, only 0·5 or 1×10^6 T8+ VV-AC were tested in the various reconstitution experiments. Full antigen-dose responses were carried out elsewhere (Lehner, 1982), so that only 1000 ng and 1 ng doses of SA were tested here. The doses of 1, 10 and 100 μl of helper or suppressor factor derived from reconstitution experiments were assayed in the cooperative cultures with CBA mouse spleen cells. DNP-SA was added to the latter in order to assess the optimum amount of specific factor, or TNP-TT to assess any non-specific factor which might have been induced.

Tests for antigen specificity of the helper and suppressor functions

The specificity of SA binding to T8+ cells (Lehner, 1983a) and those of the helper and suppressor functions (Lehner, 1982; Lehner, 1983b) have been recently established. Some of these specificities have been repeated here by antigenic stimulation of helper or suppressor functions with TT and using DNP-SA in the cooperative culture to assay antibody-forming cells. Conversely, SA was used to stimulate helper or suppressor function, and using TNP-TT was added to the cooperative cultures to assay antibody-forming cells.

The effect of monoclonal antibodies to T8 and monocytes on the helper and suppressor functions of T8+ VV-AC and monocytes

In order to confirm that the SA-presenting T8+ cells and monocytes belong to two different groups of cells, the T8+VV-AC were treated with anti-T8 antibodies and complement, or the latter alone, as described for separating T4+ cells. Monocytes were similarly treated with anti-T8 antibodies and complement and, in addition, with OK.M1 antibodies (Ortho Laboratories) and complement, or the latter alone. The treated cells were then reconstituted with T4+HC and SA, and the helper and suppressor functions were then assayed.

RESULTS

The yield and viability of VV-separated cells

The yield of VV-AC from T cells was a mean (±

standard error) of 14·8 ± 3·2%, and the yield of VV-NAC was 31·6 ± 4·8% in five subjects. The corresponding yields of the T8+ cells were higher, with 19·7 ± 3·2% of T8+ VV-AC and 48·1 ± 11·4% of T8+ VV-NAC in six subjects. The viabilities of all VV-separated cell populations were between 88% and 97%. A proportion of the cells was lost during the process of separation and this was considerably greater with T than with T8+ cells.

Phenotypic characterisation of VV-separated cells

T cells panned on VV plates (VV-AC) showed almost twice as many T8+ than T4+ cells and the converse was found with the VV-NAC (Table 1). Significant differences were found between the VV-AC as compared with VV-NAC in T8+ cells ($t = 3{\cdot}581$, *df* 6, $P < 0{\cdot}02$), T5+ cells ($t = 2{\cdot}788$, *df* 4, $P < 0{\cdot}05$) and T4+ cells ($t = 2{\cdot}670$, *df* 6, $P < 0{\cdot}05$). The proportion of Ia-positive cells was 7·8 ± 1·6% in the VV-AC, compared with 2·3 ± 0·6% in the VV-NAC ($t = 4{\cdot}138$, *df* 4, $P < 0{\cdot}02$). Neither population showed any monocytes detectable by the uptake of latex particles or B cells detectable by anti-F(ab) antiserum. The T8+ VV-AC showed 75·2 ± 2·2% T8+, 11·3 ± 1·4% Ia+, 0·2 ± 0·2% T4+ and no detectable B cells or monocytes, whereas the T8+ VV-NAC showed 66·8 ± 3·7% T8+ cells, 2·1 ± 1·0% T4+ and 3·4 ± 0·1% Ia+ cells (Table 1).

The T8+ VV-AC population showed a significantly higher proportion of Ia+ cells ($t = 5{\cdot}933$, *df* 2, $P < 0{\cdot}05$) and T8+ cells ($t = 5{\cdot}556$, *df* 2, $P < 0{\cdot}05$) than the T8+ VV-NAC. In both populations of cells, there were some null cells but their significance is doubtful, as discussed elsewhere (Lehner, 1983). The monocytes showed 71% phagocytic cells, 2·4% T8+, 1·3% F(ab)+ and 27% Ia+ cells.

Helper function of the separated and reconstituted VV-AC and VV-NAC

When the culture supernatants were assayed for the number of antibody-forming cells in the mouse spleen B-cell indicator system, neither VV-NAC nor VV-AC generated any helper function with 1 μg SA (Fig. 1) after 24 hr culture in the Marbrook-Diener flasks. Reconstitution of increasing number of VV-NAC with decreasing number of VV-AC generated optimum helper activity with the ratios of 3 VV-NAC: 2 VV-AC, or 4 VV-NAC:1 VV-AC. These ratios were similar to those resulting from reconstitution of VV-NAC with Mo (Fig. 1). Hence, VV-AC in the presence of 1 μg SA functions as effectively as monocytes in activating VV-NAC to helper activity.

Helper and suppressor functions of reconstituted T4+ HC with T8+ VV-AC or T8+ VV-NAC

The T cells were further separated to T8+ cells and these were then panned on VV plates in order to study T8+ VV-AC and T8+ VV-NAC. T4+ HC failed to generate any helper or suppressor activity (Table 2a, b). Separated T8+ VV-NAC generate little helper but 58–100% suppressor activity (Table 2a, b; Fig. 2). Reconstitution of T4+ HC with T8+ VV-NAC (4:1) had little or no effect on the helper or suppressor activity (Table 2a, b; Fig. 2). However, reconstitution of T4+ HC with T8+ VV-AC resulted in a significant increase in helper (324–680%) and no suppressor activity (Table 2a, b; Fig. 2). Three-cell reconstitution of T4+ HC with T8+ cells and T8+ VV-NAC (3:1·5:1) resulted in little help and more than 90% suppression (Table 2c; Fig. 2). However, T8+ VV-AC can reverse this by preventing the suppressor and

Table 1. Phenotypic characterization of *Vicia villosa* adherent and non-adherent T and T8+ cells

Cell population	*n*	% cells (mean ± SE) T4	T5	T8	Ia
VV-adherent T cells*	7	25·8 ± 6·2	34·2 ± 5·4†	45·0 ± 5·6	7·8 ± 1·6†
VV-non-adherent T cells	7	48·1 ± 3·6	18·5 ± 7·0†	29·1 ± 5·5	2·3 ± 0·6
VV-adherent T8+ cells	3	0·2 ± 0·2	ND‡	75·2 ± 2·2	11·3 ± 1·4
VV-non-adherent T8+ cells	3	2·1 ± 1·0	ND	66·8 ± 3·7	3·4 ± 0·1

* VV, *Vicia villosa*.
† $n = 5$.
‡ NT, not done.

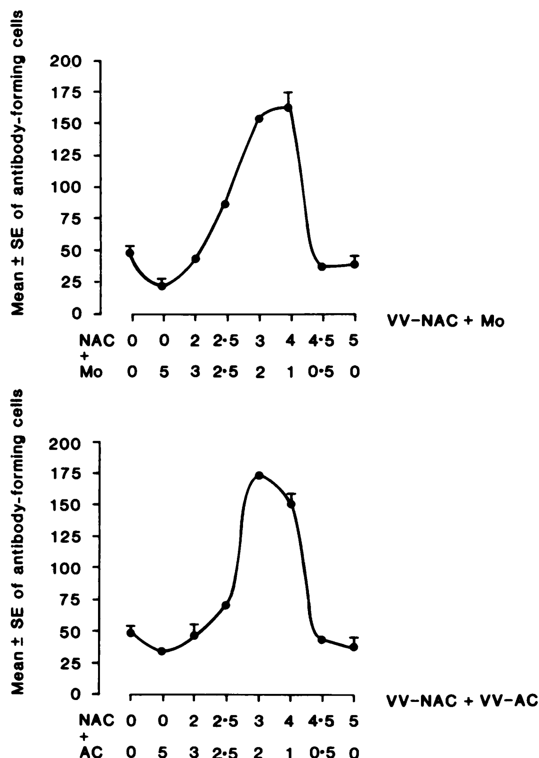

Figure 1. The effect of reconstitution of *Vicia villosa* (VV)-non-adherent cells (NAC) with VV-adherent cells (AC) or monocytes (Mo) on the helper function to 1 μg streptococcal antigen; each point is the mean $\pm$(SE) of four different subjects or of two subjects, where SE is not given.

generating 233–309% helper activity. It should be noted that $T8^+$ cells reconstituted with $T4^+$ HC resulted in 84–98% suppression, as $T8^+$ cells consist predominantly of $T8^+$ VV-NA suppressor cells (Table 2d).

The effects of varying the number of $T8^+$ VV-AC, the dose of SA and the dose of helper or suppressor factor on the corresponding function

Titration of cell numbers showed that 1×10^6 $T8^+$ VV-AC were much more effective than $0{\cdot}5 \times 10^6$ $T8^+$ VV-AC for antigen presentation and activation of $T4^+$ HC (Table 3b). In the three-cell reconstitution experiments, $0{\cdot}5 \times 10^6$ $T8^+$ VV-AC prevented suppression of $T4^+$ HC by $T8^+$ SC, but yielded only 67% helper activity, whereas 1×10^6 $T8^+$ VV-AC both prevented suppression and generated 223% helper activity (Table 3d).

The dose dependency of the $T8^+$ SA binding and presenting cells was demonstrated earlier (Lehner, 1982; Lehner, 1983a) and confirmed here. $T8^+$ VV-AC induce $T4^+$ HC to generate helper activity with 1000 ng, but not 1 ng SA (Table 3b). On the other hand, $T8^+$ VV-NAC will induce little or no helper activity of $T4^+$ HC with either 1000 ng or 1 ng SA (Table 3c), although both doses of SA will induce a high degree of suppressor activity (Table 3a). Furthermore, in the three-cell reconstitution experiments, 1 ng failed to prevent suppresion by the $T8^+$ VV-AC, unlike 1000 ng SA (Table 3d).

The optimal dose of helper factor derived from reconstitution of $T4^+$ HC with $T8^+$ VV-AC was 10 μl (Table 4a). Little or no non-specific helper activity tested with TNP-TT was detected with any of three doses of helper factor. Similarly, reconstitution of $T4^+$ HC with $T8^+$ cells and $T8^+$ VV-AC to test for contrasuppressor function again showed only SA-specific helper activity which was optimal with 10 μl of helper factor (Table 4b). Reconstitution of $T4^+$ HC with $T8^+$ cells and $T8^+$ VV-NAC to test for suppressor activity showed optimal SA-specific suppression with 10 and 100 μl suppressor factor, but no detectable non-specific suppression with any of the three doses of suppressor factor (Table 4c).

Antigen specificity of the helper and suppressor functions

The specificity tests for helper and suppressor functions showed that antigenic stimulation with TT in the initial culture for 24 hr and using DNP-SA in the cooperative culture failed to induce significant helper or suppressor function, as compared with stimulation by SA in both cultures (Table 5a, b, c). Conversely, stimulation with SA in the initial culture and with TNP-TT in the cooperative culture also failed to induce helper or suppressor function, as compared with that induced by DNP-SA in the various reconstitution experiments (Table 5, d–g).

The effect of killing with monoclonal antibodies to $T8^+$ cells and to monocytes on the helper and suppressor functions of $T8^+$ VV-AC and monocytes

The helper function was almost lost when $T4^+$ HC were reconstituted with $T8^+$ VV-AC treated with anti-T8 antibodies and complement (65%), as com-

Table 2. The effects of reconstitution of T4⁺ HC with either T8⁺VV-AC or T8⁺ VV-NAC and 1 μg SA on the helper and suppressor function

Cell reconstitutions	Cell ratio ($\times 10^6$)	Antibody-forming cells ± SE		Percent	
		Help	Suppression	Help	Suppression
(a) DNP-SA* (Control)		33±3	33±3		
Mouse HC†		163±13			
$T4^+$ HC	5	30±6	183±22	0	0
$T8^+$ VV-NAC‡	5	30±15	33±9	0	100
$T4^+$ HC+$T8^+$ VV-AC§	4:1	140±25	167±15	324	0
$T4^+$ HC+$T8^+$ VV-NAC	4:1	53±9	103±9	61	46
(b) DNP-SA (Control)		30±6	33±3		
Mouse HC		157±7			
$T4^+$ HC	5	43±12	—	16	
$T8^+$ VV-NAC	5	37±18	87±9	23	58
$T4^+$ HC+$T8^+$ VV-AC	4:1	133±9	143±19	343	13
$T4^+$ HC+$T8^+$ VV-NAC	4:1	23±9	133±9	0	21
(c) DNP-SA (Control)		22±9	27±9		
Mouse HC		137±12			
$T4^+$ HC+$T8^+$ C¶+$T8^+$ VV-AC	3:1·5:1	90±20	130±12	309	10
$T4^+$ HC+$T8^+$ C+$T8^+$ VV-NAC	3:1·5:1	30±12	33±13	36	95
(d) DNP-SA (Control)		23±9	17±3		
Mouse HC		140±6			
$T4^+$ HC+$T8^+$ C¶	3:1·5	27±7	30±13	17	84
$T4^+$ HC+$T8^+$ C	3:2·0	20±6	20±6	0	98

* SA, streptococcal antigen.
† HC, helper cells.
‡ VV, *Vicia villosa*; NAC, non-adherent cells.
§ AC, adherent cells.
¶ C, cells.

pared with complement alone (660%) (Table 6). In contrast, the helper function of $T4^+$ HC, reconstituted with monocytes which were treated with anti-T8 antibodies and complement, was unchanged (from 635 to 658%), whereas monocytes treated with the OK.M1 antibody and complement lost most of the helper function (from 635% to 115%). There was no change in the minimal or no suppression. These results support the view that the responding populations of $T8^+$ cells and monocytes are independent and not due to a small proportion of contaminating $T8^+$ cells among the monocytes or the latter among the $T8^+$ cells.

DISCUSSION

The lectin *Vicia villosa* separated T8 cells into a small VV-AC and a large VV-NAC subset. The separated T VV-AC or VV-NAC are not induced by SA alone to generate helper function. However, reconstitution of the two subsets showed that a ratio of 4:1 or 3:2 of VV-NAC to VV-AC induces an increase in direct antibody-forming cells (Fig. 1). A comparison of the helper function generated by reconstituted VV-NAC and VV-AC with that of reconstituted VV-NAC and Mo showed that the optimal ratio of VV-NAC:Mo was 4:1 or 3:2, which was identical to that found for the ratio of VV-NAC to VV-AC. It is suggested that the VV-AC can present antigen and activate HC to generate helper-factor activity.

Reconstitution of $T4^+$ HC with $T8^+$ VV-AC and SA generate significant helper activity (Fig. 2). $T8^+$ VV-NAC have little or no effect on the $T4^+$ HC function. Contrasuppression was demonstrated by reconstitution of $T4^+$ HC and $T8^+$ cells with $T8^+$ VV-AC which induced helper but no suppressor

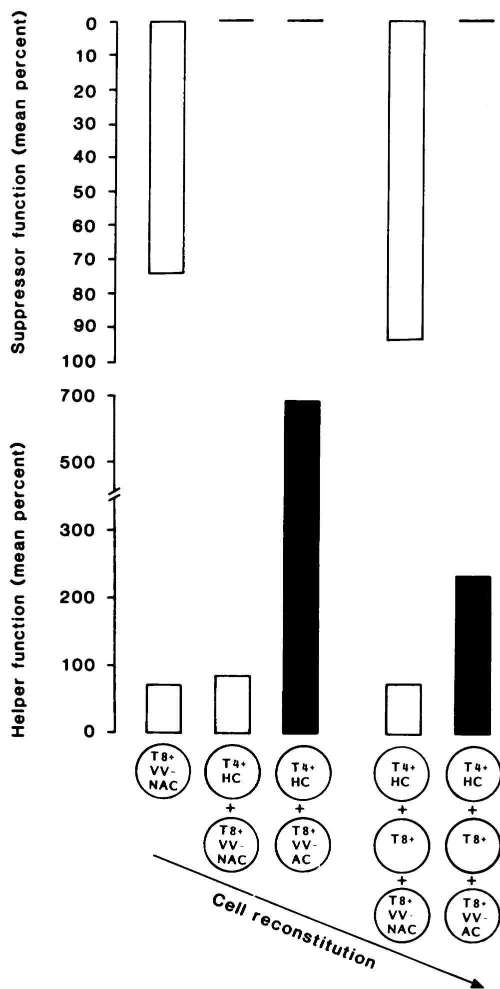

Figure 2. Parallel assays of helper and suppressor functions induced by 1 μg steptococcal antigen and T4$^+$ helper cells (HC), reconstituted with T8$^+$ *Vicia villosa* (VV)-non-adherent cells (NAC) or T8$^+$ VV-adherent cells (AC) at a ratio of 4:1 × 10^6 cells. T4$^+$ HC were also reconstituted with unseparated T8$^+$ cells to which either T8$^+$ VV-NAC or T8$^+$ VV-AC were added (ratio of 3:1·5:1). The cells were cultured for 24 hr with 1 μg of the streptococcal antigen, and the putative helper or suppressor factor was assayed in the cooperative cultures with mouse spleen cells as described in the text.

function. However, substitution of T8$^+$ VV-NAC for T8$^+$ VV-AC resulted in little help and greater than 90% suppression.

The antigen specificity of the helper and suppressor assays were established previously (Lehner, 1982; Lehner *et al.*, 1981; Lehner, 1983b). This was confirmed here with the T8$^+$ VV-AC and T8$^+$ VV-NAC by using either TT in generating the helper or suppressor factors and DNP-SA in the cooperative culture, or SA in generating the factors and TNP-TT in the cooperative culture (Table 5). Helper or suppressor activity was generated with SA only when DNP-SA was used in the cooperative culture. The possibility that non-specific factors might have been generated when larger quantities of helper or suppressor factors had been assayed cannot be entirely excluded. However, up to 100 μl of the factors per culture yielded no or little non-specific activity, whereas 10 μl yielded optimal and even 1 μl significant specific helper activity (Table 4).

The mechanism whereby the T8$^+$ VV-AC present antigen to T4$^+$ HC and activate them to generate helper and contrasuppressor functions is unknown. It is, however, likely that some of the Ia determinants might be involved, as the proportion of Ia$^+$ cells is significantly greater in the VV-AC than the VV-NAC population (Table 1), and killing with monoclonal anti-Ia antibody and complement significantly reduces the helper and contrasuppressor functions (R. Brines and T. Lehner, manuscript in preparation).

It should be noted that the T8$^+$ VV-AC differ from monocytes in their phenotypic expression, with 75·2±2·2% T8$^+$, 11·3±1·4% Ia$^+$ and no detectable phagocytic or F(ab)$^+$ cells, whereas monocytes showed 71% phagocytic, 2·4% T8$^+$ and 27% Ia$^+$ cells. Morphological analysis with acid naphthyl acetate esterase revealed that the monocytes showed almost exclusively diffuse cytoplasmic staining, whereas the T8$^+$ cell showed a punctate pattern (T. Lehner, unpublished observations). Monocytes bind ^{125}I-SA directly at 37°, but not at 4°, whereas T8$^+$ cells bind ^{125}I-SA at 4° as effectively as at 37° (Lehner & Jones, 1984). Furthermore, SA binding of T8$^+$ cells, unlike monocytes, is inhibited by monoclonal anti-T8 antibodies and by anti-suppressor factor antiserum. Depletion studies confirmed the independent nature of the two cell populations; treatment with anti-T8 antibodies and complement virtually abolished the helper activity of T8$^+$ VV-AC, but had no effect on monocytes (Table 6). However, treatment of monocytes with OK.M1 antibodies and complement resulted in the loss of most of the helper activity.

The rationale for using VV was that the lectin binds murine CSC (Green *et al.*, 1981). It seems, therefore, that VV binds human, as well as murine, CSC, so that they may share some cell surface determinants, of

Table 3. The effects of cell number and the dose of streptococcal antigen (SA) on reconstitution of T4+ HC with T8+ VV-AC and T8+ VV-NAC on the helper or suppressor function

Cell reconstitutions	Cell ratio (×10^6)	Dose of SA(ng)	Antibody-forming cells (mean±SE) Help	Antibody-forming cells (mean±SE) Suppression	Percent Help	Percent Suppression
DNP-SA (control)			27±3	27±7		
Mouse HC			148±6			
(a) T8+ VV-NAC	5	1000	47±9	57±9	74	75
T8+ VV-NAC	5	1	33±12	37±23	22	92
(b) T4+ HC+T8+ VV-AC	4·5:0·5	1000	93±3	146±12	244	2
T4+ HC+T8+ VV-AC	4:1	1000	213±15	157±26	689	0
T4+ HC+T8+ VV-AC	4:1	1	37±12	167±23	37	0
(c) T4+ HC+T8+ VV-NAC	4:1	1000	50±6	160±15	85	0
T4+ HC+T8+ VV-NAC	4:1	1	10±6	146±3	0	0
DNP-SA (control)			30±6	33±3		
Mouse HC			157±7			
(d) T4+ HC+T8+ C+T8+ VV-AC	3:1·5:0·5	1000	50±10	190±21	67	0
T4+ HC+T8+ C+T8+ VV-AC	3:1·5:1	1000	97±12	153±23	223	3
T4+ HC+T8+ C+T8+ VV-AC	3:1·5:0·5	1	17±3	53±12	0	84

Abbreviations as for Table 2.

Table 4. The effect of dose of factors derived from reconstituted T4+ HC and T8+ VV-AC, with or without T8+ cells and with SA on the helper and suppressor functions of DNP-SA of TNP-TT stimulated spleen cells

			Helper activity with:				Suppression with:			
			DNP-SA		TNP-TT*		DNP-SA		TNP-TT	
Cell reconstitution	Ratio	Factors (μl)	AFC†	%	AFC	%	AFC	%	AFC	%
DNP-SA (control)			27±3		27±7		23±3		30±6	
Mouse HC			123±15							
(a) T4+ HC+T8+ VV-AC	4:1	1	43±12	59	27±7	0	ND‡		ND	
		10	123±15	356	23±9	0	ND		ND	
		100	65±9	141	40±10	48	ND		ND	
(b) T4+ HC+T8+ C+T8+ VV-AC	3:1·5:1	1	90±15	233	33±3	22	ND		ND	
		10	163±9	504	27±3	0	ND		ND	
		100	97±15	259	30±6	11	ND		ND	
(c) T4+ HC+T8+ C+T8+ VV-NAC	3:1·5:1	1	ND		ND		77±9	44	137±12	0
		10	ND		ND		47±7	75	127±13	0
		100	ND		ND		43±12	80	140±15	0

Abbreviations as for Table 2.
* TT, tetanus toxoid.
† AFC, antibody-forming cells.
‡ ND, not done.

which N-acetyl-D-galactosamine might be of special significance. The VV used has a high binding affinity for N-acetyl-D-galactosamine, and the T8+ VV-AC were recovered by treatment with N-acetyl-D-galactosamine, so that binding of T8+ cells to VV might be associated with N-acetyl-D-galactosamine residues. However, the role of N-acetyl-D-galactosamine in antigen binding of the T8+ cell needs to be further explored.

The significance of an antigen-presenting cell which

Table 5. The specificity of helper and suppressor functions in cell reconstitution experiments

Cell reconstitutions	Cell ratio ($\times 10^6$)	Help AFC±SE	Suppression AFC±SE	Help (%)	Suppression (%)
DNP-SA (control)		33±3	33±3		
Mouse HC		163±13			
(a) $T8^+$ VV-NAC+SA	5	30±15	33±9	0	100
$T8^+$ VV-NAC+TT	5	57±12	147±9	73	12
(b) $T4^+$ HC+$T8^+$ VV-AC+SA	4:1	140±25	167±15	324	0
$T4^+$ HC+$T8^+$ VV-AC+TT	4:1	33±9	140±12	0	18
(c) $T4^+$ HC+$T8^+$ VV-NAC+SA	4:1	53±9	103±9	61	46
$T4^+$ HC+$T8^+$ VV-NAC+TT	4:1	43±7	163±9	30	0
DNP-SA (control)		22±9	27±9		
TNP-TT (control)		40±6	40±6		
Mouse HC		137±12			
(d) $T4^+$ HC+$T8^+$ VV-AC+SA (SA)	4:1	137±15	157±26	523	0
$T4^+$ HC+$T8^+$ VV-AC+SA (TT)	4:1	50±10	130±0	25	7
(e) $T4^+$ HC+$T8^+$ VV-NAC+SA (SA)	4:1	50±6	30±6	127	97
$T4^+$ HC+$T8^+$ VV-NAC+SA (TT)	4:1	33±12	133±7	0	4
(f) $T4^+$ HC+$T8^+$ C+$T8^+$ VV-AC (SA)	3:1·5:1	90±20	130±12	309	10
$T4^+$ HC+$T8^+$ C+$T8^+$ VV-AC (TT)	3:1·5:1	23±9	133±23	0	4
(g) $T4^+$ HC+$T8^+$ C+$T8^+$VV-NAC (SA)	3:1·5:1	30±12	33±13	36	95
$T4^+$ HC+$T8^+$ C+$T8^+$ VV-NAC (TT)	3:1·5:1	27±3	147±15	0	0

The specificity of helper and suppressor functions in cell reconstitution experiments of $T4^+$ HC with $T8^+$ VV-AC or $T8^+$ VV-NAC was tested by using either SA or TT in the priming cultures and DNP-SA in the cooperative cultures (exps a–c) or SA in the priming and DNP-SA (SA) or TNP-TT (TT) in the cooperative cultures (exps d–g).
Abbreviations as for Table 2.

Table 6. The effect of treatment of $T8^+$ VV-AC or monocytes with McAb OK.T8 or OK.M1 and complement on the helper and suppressor functions when 1×10^6 of these cells were reconstituted with 4×10^6 $T4^+$ HC and 1 μg SA

Cell reconstitutions (and treatment)	Help AFC*±SE	Suppression AFC±SE	Help (%)	Suppression (%)
DNP-SA (control)	20±0	20±0		
Mouse HC	157±3			
$T4^+$ HC+$T8^+$ VV-AC (OK.T8+C′)†	33±9	170±10	65	0
$T4^+$ HC+$T8^+$ VV-AC (C′)	152±11	150±20	660	5
$T4^+$ HC+monocytes (OK.T8+C′)	157±13	143±20	685	10
$T4^+$ HC+monocytes (OK.M1+C′)	43±7	137±15	115	15
$T4^+$ HC+monocytes (C′)	147±9	157±20	635	0

Abbreviations as for Table 2.
* AFC, antibody-forming cells.
† C′, complement.

also has a contrasuppressor function may reside in effectively countering a dominant suppressor function. If the target of the suppressor cell (or factor) is the $T4^+$ HC, then there is considerable biological advantage for a subset of $T8^+$ cells to incorporate both functions of antigen presentation and prevention of suppression. However, the possibility that these functions are performed by two subsets of $T8^+$ VV-AC cannot be excluded. Contrasuppression may play a significant role in autoimmunity (Smith *et al.*, 1982) in

which suppression may exist, but its function is prevented by the activity of contrasuppressor cells, and this may result in boosting the helper immune response.

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